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Shedding of foodborne pathogens by *Caenorhabditis elegans* in compost-amended and unamended soil

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Abstract

A study was done to characterize the shedding of foodborne pathogenic bacteria by *Caenorhabditis elegans*, evaluate the persistence of worm populations cocultured with foodborne pathogens, and determine if *C. elegans* disperses ingested pathogens in soil as a result of shedding. *Escherichia. coli* O157:H7, *Salmonella enterica* serotype Poona, and *Listeria monocytogenes*, as well as *E. coli* OP50, a non-pathogenic strain, were studied. Synchronous populations of *C. elegans* were fed for 24 h on confluent lawns of nalidixic acid-adapted bacteria. *C. elegans* shed viable cells of ingested bacteria on tryptic soy agar supplemented with nalidixic acid ($50\,\mu g\,ml^{-1}$) (TSAN) throughout a 5-h post-feeding period. *C. elegans* persisted for up to 10 days by feeding on bacteria that had been shed and grew on TSAN. Eggs harvested from *C. elegans* cultured on shed foodborne pathogens had the same level of viability as those collected from *C. elegans* grown on shed *E. coli* OP50. After 6–7 days, 78%, 64%, 64%, and 76% of eggs laid by *C. elegans* that had fed on *E. coli* O157:H7, *S.* Poona, *L. monocytogenes*, and *E. coli* OP50, respectively, were viable. Worms fed on *E. coli* O157:H7 were inoculated into soil and soil amended with turkey manure compost. Populations of *C. elegans* persisted in compost-amended soil for at least 7 days but declined in unamended soil. *E. coli* O157:H7 was detected at 4 and 6 days post inoculation in compost-amended and unamended soil, and in unamended soil inoculated with *E. coli* OP50. Populations of *E. coli* O157:H7 in soil amended with turkey manure compost were significantly($\alpha = 0.05$) higher than those in unamended soil. Results indicate that *C. elegans* can act as a vector to disperse foodborne pathogens in soil, potentially resulting in increased risk of contaminating the surface of pre-harvest fruits and vegetables.

Keywords: Nematode; *Caenorhabditis elegans*; *Escherichia coli* O157:H7; *Salmonella enterica*; *Listeria monocytogenes*; Soil contamination

1. Introduction

The number of documented outbreaks of human infections associated with the consumption of raw fruits and vegetables has increased in the United States in recent years. Outbreaks with identified etiology are predominantly of bacterial origin (Beuchat, 2002; Buck et al., 2003; Mead et al., 1999; NACMCF, 1999). *Salmonella*, *Escherichia. coli* O157:H7, and *Shigella* have been identified as causative agents in outbreaks of enteric infections associated with fresh produce. Listeriosis has been linked to the consumption of foods of animal origin but the causative agent, *Listeria monocytogenes*, can be found in decaying vegetation in soil, which serves as a source of contamination for produce (Beuchat, 1996). *Salmonella*, *E. coli* O157:H7, and *L. monocytogenes* have been reported to persist in soil for

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extended periods (Beuchat, 2002; Nicholson et al., 2000). A major source of *Salmonella* and *E. coli* O157:H7 in soils is excrement from wild and domestic animals. Agricultural practices that use improperly treated manure or compost for amending soil or allow animals to graze in or near fields used to grow fruits and vegetables, may contribute to higher risks of contamination by foodborne pathogens.

Enteric pathogens have been reported to internalize tissues of pre-harvest lettuce (Solomon et al., 2002; Wachtel et al., 2002), alfalfa sprouts (Gandhi et al., 2001), cress (Cooley et al., 2003), and tomatoes (Guo et al., 2002). Sources and persistence of these pathogens are influenced by agronomic practices (Nicholson et al., 2000). An understanding of environmental factors that affect the population size, distribution, and persistence of foodborne pathogens in soil as affected by these practices is essential to assessing the risk of pre-harvest contamination of fruits and vegetables.

Nematodes are the most abundant soil metazoa and play an important role in soil ecology. Free-living forms that feed on bacteria respond rapidly to new nutrient sources (Bongers and Bongers, 1998) and have a major impact on soil micro-fauna. Free-living nematodes harbor ingested bacteria, including human pathogens (Aballay et al., 2000; Anderson et al., 2003; Caldwell et al., 2003a, b; Kenney et al., 2005). Populations of bacterivorous nematodes increase in manure-amended soils (Freckman and Caswell, 1985; Mikola and Sulkava, 2001; Opperman et al., 1989; Valocka et al. 2000; Wasilewska and Webster, 1975). The role of nematodes in transporting bacteria and as disease vectors in soil has been described (Cayrol et al., 1977; Knox et al., 2003; Stephens et al., 1993; Wasilewska and Webster, 1975). Thus, the potential for nematodes to act as vectors of human pathogenic bacteria may be increased if manure or improperly treated compost are used as soil amendments.

C. elegans, an extensively studied free-living soil nematode, feeds on a broad spectrum of bacterial species, including pathogens implicated in human disease (Caldwell et al, 2003b; Kurz and Ewbank, 2000). Caldwell et al. (2003b) demonstrated that *C. elegans* populations survive for up to 7 days when fed with *Salmonella* and *E. coli* O157:H7. Pathogens ingested by nematodes are protected against treatment with chlorinated water (Chang et al., 1960; Kenney et al., 2004; Walters and Holcomb, 1967). Protection of *Salmonella* (Caldwell et al., 2003b; Kenney et al., 2004, 2004b) and *E. coli* O157:H7 (Kenney et al., 2005) ingested by *C. elegans* against produce sanitizers has been described, and viable pathogens are associated with both live and dead worms for some time after exposure. Thus, nematodes represent a potential reservoir for foodborne pathogens that could contribute to pre-harvest contamination of fruits and vegetables.

We conducted a study to determine the time course over which *C. elegans* sheds ingested *E. coli* O157:H7, *Salmonella enterica* serotype Poona, *L. monocytogenes*, and a non-virulent strain (OP50) of *E. coli*. The ability of *C. elegans* to survive and reproduce on these bacteria was determined. The effect of adding turkey manure compost to soil on populations of *C. elegans* and *E. coli* O157:H7 ingested by worms before inoculation of soil was studied.

2. Materials and methods

2.1. Maintenance of C. elegans

C. elegans N2 wild-type strain and a transgenic strain (CB5584) that strongly expresses green fluorescent protein (GFP) in the pharynx region, obtained from the Caenorhabditis Genetics Center (Minneapolis, Minnesota, USA), were used in agar and soil experiments, respectively. Both strains were maintained as described by Donkin and Williams (1995) to provide age-synchronized worms. Briefly, eggs were harvested from K-agar plates (Williams and Dusenbery, 1988), incubated for 3 days at 20 °C and rinsed with a 1% NaOCl solution prepared by diluting Clorox® (Clorox Co., Oakland, California, USA) with water to kill all life stages except eggs. Eggs rinsed in K-medium (Williams and Dusenbery, 1990) were used to inoculate lawns of *E. coli* strain OP50 established by surface inoculating K-agar with 0.1 ml of a 24-h culture of the bacterium and incubating for 24 h at 20 °C. Worms were cultured for 2 days at 20 °C, collected, and washed two times in K-medium prior to transfer to tryptic soy agar (TSA, pH 7.2) (Difco/BBL, Sparks, Maryland, USA) for culturing on lawns of bacterial pathogens and *E. coli* OP50.

2.2. Pathogens used and preparation for use as food source

Bacterial pathogens adapted to grow in tryptic soy broth (TSB, pH 7.2) (Difco/BBL) containing nalidixic acid (50 μg ml^{-1}) (TSBN) were used. *S. enterica* serotype Poona (strain 01A4754, from patient with salmonellosis associated with consuming cantaloupe), enterohemorrhagic *E. coli* O157:H7 (strain SEA-13B88, from patient infection associated with consuming apple cider), and *L. monocytogenes* (strain G1091, from a patient in a coleslaw-associated outbreak of listeriosis), and a non-virulent strain of *E. coli* OP50 (control) were grown in TSBN at 20 °C and maintained as stock cultures at 4 °C. Inocula (0.1 ml) of 24-h cultures incubated at 37 °C were spread on TSA supplemented with nalidixic acid (50 μg ml^{-1}) (TSAN) and incubated at 37 °C for 24 h. *C. elegans* that had fed on these bacterial lawns for 24 h

at 20 °C was used to assess reproductive behavior and shedding characteristics upon transfer to fresh TSAN and soil.

2.3. Shedding of ingested bacteria

Synchronous populations of worms cultured for 2 days at 20 °C on a confluent lawn of *E. coli* OP50 on K-agar were transferred to TSAN plates on which lawns of *E. coli* O157:H7, *S.* Poona, *L. monocytogenes*, or *E. coli* OP50 had formed. After a 24-h feeding period, worms were collected, washed three times with 5 ml of sterile K-medium, and placed on sterile agar. Pairs of 3-day-old adult worms were collected from the agar surface in 5 µl of K-medium at times ranging from 0 to 5 h and transferred to TSAN plates. After 3 h at 20 °C, worms were killed with a hot wire and plates were incubated at 37 °C for 24 h. Colonies formed by bacteria that had been shed by *C. elegans* were counted. Experiments were replicated three to six times.

2.4. Population of C. elegans fed on pathogenic bacteria and the viability of eggs

Synchronous populations of *C. elegans* were fed on foodborne pathogens or *E. coli* OP50 for 24 h at 20 °C as described above. Young adult worms, washed three times with 5 ml of sterile K-medium, were held for 2 h at 20 °C on TSAN in the absence of an exogenous food source. Pairs of worms were then transferred to sterile TSAN (six to eight plates for each of the four test bacteria). Worms were fed on shed bacteria for a 9-day period. Populations of worms and the number of colonies formed by test bacteria were monitored over a 10-day period at 20 °C. Plates were scored as positive or negative for presence of bacterial colonies, viable worms, and eggs. The frequency of occurrence of worms and eggs was calculated as number of positive cultures per number of plates examined.

Eggs laid by second- or third-generation adults were collected from cultures after incubating plates for 5, 6.5, and 10 days. Worms and eggs were collected in K-medium and treated with 1% NaOCl solution at 20 °C for 12 min to kill adult and larval stage worms. Eggs were washed two times in 8 ml of sterile K-medium. Suspensions (5–10 µl) containing 10–30 eggs were transferred to wells of single-depression culture microscope slides. The final volume in each well was brought to 15–20 µl with K medium and the slides were placed in a closed high-humidity environment to prevent desiccation. After 18–20 h at 20 °C, samples were examined microscopically. The number and percent of eggs that hatched were calculated. All calculations were based on duplicate samples of each treatment and all treatments were replicated three to six times.

2.5. Contamination of soil by ingested bacteria

C. elegans (strain CB5584) was fed on confluent lawns of *E. coli* O157:H7 on TSAN for 24 h at 20 °C. Worms were collected and washed three times in 15 ml of K-medium to remove loosely adherent cells on their cuticle before inoculating into Tifton soil (pH 5.14) (Boyd et al., 2003; Peredney and Williams, 1999), which is a sandy loam soil (organic carbon, 6.7 g kg^{-1}; cation exchange capacity, 1.58 cmol^{+} kg^{-1}; clay, 36 g kg^{-1}; sand, 886 g kg^{-1}) collected from the lower Piedmont region of Georgia, and into soil amended with turkey manure compost. The number of *E. coli* O157:H7 cells that remained on the washed worms was very small compared to the number of viable cells located in the gut of *C. elegans.* Soil (2.3 g) moistened with 0.8 ml of deionized water and moistened soil amended with turkey manure compost (0.66%, wt/wt) provided by the USDA-Agricultural Research Service, Beltsville, Md. were inoculated with worms that had fed on *E. coli* O157:H7. Worms that had fed on *E. coli* O157:H7 were also inoculated into compost-amended soil that had been inoculated with a 24-h culture of *E. coli* OP50 to give a population of 6.6 $\log_{10}$ cfu g^{-1} of soil.

Seven 3-day-old adult worms were inoculated into each soil system. Inoculated soils in 35-mm diameter petri plates were sealed with Para-film to prevent desiccation during the course of incubation at 20 °C for 7 days. Soil was examined for populations of *E. coli* O157:H7 at 4 and 6 days, and for *C. elegans* at 3, 5, and 7 days. To determine the population of *E. coli* O157:H7, samples of cultures were serially diluted in K-medium and plated on TSAN. Colonies formed within 24 h at 37 °C were counted and confirmed to be *E. coli* O157:H7 using a *E. coli* O157 agglutination test (Oxoid, Basingstoke, UK) and the API 20E diagnostic kit (bioMerieux-Vitek, Inc., Hazelwood, Mo.). Populations of *C. elegans* were estimated by direct counts of viable (i.e. spontaneously moving) fluorescent worms viewed under ultraviolet illumination at 28 × magnification. Counts are presented as the number of worms visually observed in five non-overlapping fields. This procedure enabled inspection of approximately 50% of the total soil or compost-amended soil volume. Experiments were replicated three times.

3. Results and discussion

Studies using *C. elegans*, a rhabitid nematode that feeds on soil bacteria, as a model host organism have contributed to understanding the relationship between selected human pathogenic bacteria and nematodes (Aballay et al., 2000; Kurz and Ewbank, 2000). Still, relatively little has been reported on the interactions between pathogens known to be involved with

foodborne disease and free-living bacterivorous nematodes. Anderson et al. (2003) reported that *C. elegans* feeds on and disperses bacteria, including surrogate species closely related to foodborne pathogens. Caldwell et al. (2003a) demonstrated that *C. elegans* was attracted to seven strains of *E. coli* O157:H7, eight serotypes of *Salmonella*, and six strains of *L. monocytogenes* in the absence of an alternative food source. Nematode populations persisted for 7 days during which these pathogens were the dominate if not the sole food source. These studies also demonstrated that *C. elegans* dispersed *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* on an agar medium (Anderson et al., 2003) and *S.* Poona in soil (Caldwell et al., 2003b).

In the study reported here, we evaluated the time course over which *C. elegans* dispersed viable cells of *E. coli* O157:H7, *S.* Poona, and *L. monocytogenes* on TSAN. After feeding for 24 h on test pathogens or *E. coli* OP50, the number of colony-forming units (cfu) of bacteria shed on agar was monitored over a 5-h period (Fig. 1). *C. elegans* shed cells of all test bacteria throughout the post-exposure period. Shedding decreased between each interval of measurement following exposure, but continued for 5 h. The number ($\log_{10}$ cfu worm^{-1}) shed within 2 min after depositing on TSAN was (mean ± SD) 1.24 ± 0.54, 1.23 ± 1.01, 1.19 ± 0.94 and 0.59 ± 0.56 for *E. coli* OP50, *E. coli* O157:H7, *S.* Poona, and *L. moncytogenes*, respectively. During the first 3 h, shedding decreased markedly for all test bacteria, following first-order kinetics for *E. coli* OP50 (Fig. 1a–c), *E. coli* O157:H7 (Fig. 1a), *S.* Poona (Fig. 1b), and *L. monocytogenes* (Fig. 1c). Correlation coefficients of regression for $\log_{10}$ cfu shed verses post exposure time ranged between 0.962 and 0.995, and the half-lives calculated for clearance of ingested bacteria were 1.43, 2.28, 1.70, and 0.85 h for *E. coli* OP50, *E. coli* O157:H7, *S.* Poona, and *L. monocytogenes*, respectively. Between 3 and 5 h, clearance of ingested bacteria was no longer a dominant process, and the number of viable cells of each bacterium shed at 5 h was similar to that observed 3 h after an exposure.

Results suggest that *C. elegans*, after ingesting foodborne pathogens, may harbor cells internally and perhaps in lower numbers on the cuticle surface, and disperse cells for extended periods. The persistence of bacteria in the digestive tract of bacterivorous nematodes has been reported both for non-pathogenic *E. coli* (Garigan et al., 2002), as well as for *E. coli* O157:H7 and salmonellae (Kenney et al., 2005). Some pathogens colonize the gut of *C. elegans* and grow for extended periods. *S. enterica* serotype Typhimurium, for example, was reported to grow for several days following ingestion by *C. elegans* (Aballay et al., 2000). Studies have also shown that *Enterobacter faecalis* can establish a persistent infection in the gut of *C. elegans* (Garsin et al., 2001). For pathogens that infect nematodes, worms may harbor viable cells and be potential sources of contamination throughout their life span.

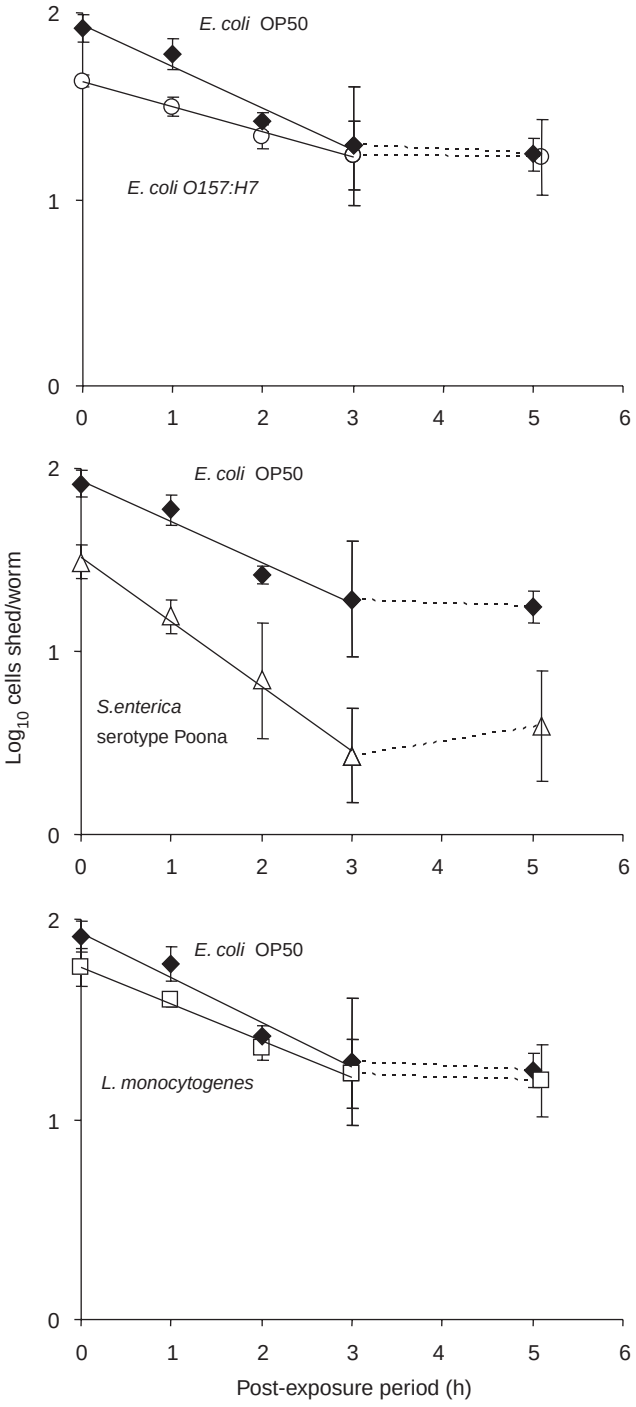

Fig. 1. Shedding of bacteria ingested by *C. elegans* as a function of post-exposure period. Symbols indicate the number ($\log_{10}$ cfu worm^{-1}) of *E. coli* OP50 (◆), *E. coli* O157:H7 (○), *S. enterica* serotype Poona (△), and *L. monocytogenes* (□) shed during a 5-h period after exposure of *C. elegans* to respective bacteria. Solid lines show linear regressions (1–3 h post exposure); dotted lines indicate point-to-point connections.

Studies using *C. elegans* as a model for evaluating host/pathogen relationships demonstrate that rhabitid

nematodes ingest and survive for extended periods on human enteric pathogens (Kurz and Ewbank, 2000; Labrousse et al., 2000; Tan and Ausubel, 2000). In addition, Caldwell et al. (2003b) reported that *C. elegans* can persist for up to 7 days feeding on strains of *E. coli* O157:H7 and *L. monocytogenes,* as well as on some serotypes of *S. enterica*. Gibbs et al. (2005) observed that *Diploscapter,* a bacterivorous free-living nematode residing in agricultural soils and compost, survives for up to 10 days feeding on foodborne pathogens, indicating that the proclivity of free-living nematodes to ingest and derive nutrients from bacteria known to cause human diseases is not restricted to only *C. elegans*.

In the current study, worms were fed for 24 h on bacteria, maintained for 2 h on sterile K agar, and then transferred to TSAN plates to assess development of colonies formed by shed bacteria and changes in worm populations. Each TSAN plate was inoculated with two 3-day-old adult worms. Colonies formed by bacteria shed by worms were evident on all plates within 2 days after inoculation and persisted throughout a 9-day post inoculation period. Second-generation adults began appearing 3 days after inoculation, and were typically present on at least 50% of the TSAN plates 6–9 days after inoculation with the four test bacteria (Fig. 2). The population of second-generation adults peaked at 8 days for *E. coli* OP50 and *S.* Poona, 6 days for *E. coli* O157:H7, and at 6–7 days for *L. monocytogenes*. The percentages of occurrence of adults (number of plates positive for adults/number of plates inoculated $\times 100$) were 83, 100, 67, and 83% on plates 8 days after inoculation with worms fed *E. coli* OP50, *S.* Poona, *E. coli* O157:H7, and *L. monocytogenes*, respectively. These results confirm the observation of Caldwell et al. (2003b), who showed that *C. elegans* survived by feeding on foodborne pathogens, and extends this work by quantifying survival of *C. elegans* fed on the three pathogens for 10 days.

An important end point demonstrating reproductive capacity of *C. elegans* is the ability of adult worms to produce viable eggs while feeding on foodborne pathogens. As shown in Fig. 2, *C. elegans* produced eggs on TSAN plates 5 days after inoculation with two adult worms, regardless of the bacterium on which it had fed. The percentage of plates positive for eggs increased through day 8, at which time the 83%, 50%, 83%, and 83% of the plates on which worms had fed on *E. coli* OP50, *E. coli* O157:H7, *S.* Poona, or *L. monocytogenes*, respectively, contained eggs. Eggs collected over a 10-day incubation period were examined for viability by assessing their ability to hatch. Results are summarized in Table 1. Eggs produced by *C. elegans* during a 10-day period after feeding on the four bacteria had similar hatchability, regardless of the bacterial species or strain, although percentages were lower for eggs from worms fed on *L. monocytogenes.* The percentage of eggs that

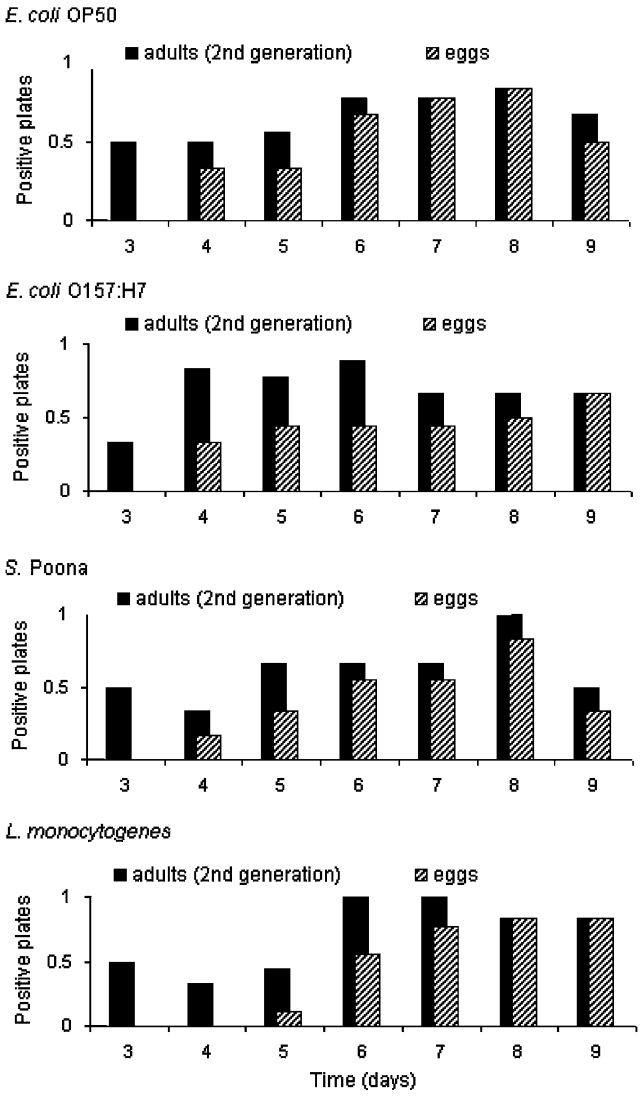

Fig. 2. Persistence (mean number of positive plates out of one plate examined in each of three to six replicate experiments) of *C. elegans* fed on colonies of *E. coli* OP50, *E. coli* O157, *S.* Poona, or *L. monocytogenes* formed by shed cells. TSAN plates were inoculated with *C. elegans* (2 worms/plate) that had fed on test bacteria. After 3 h at 20 °C, worms were killed and plates were incubated for 24 h. Plates positive for second-generation adults and eggs were monitored for 9 days.

Table 1
Viability of eggs (% that hatched) produced by *C. elegans* feeding on *E. coli* OP50 and foodborne pathogens

Bacterium	Days after feeding (% of eggs that hatched)[a]		
	5	6–7	10
E. coli OP50	59±7.3 (3)	76±9.3 (5)	69±14.2 (3)
E. coli O157:H7	70±9.0 (4)	78±10.8 (4)	53±18.0 (3)
S. Poona	60±13.1 (4)	64±9.3 (6)	71±18.0 (3)
L. monocytogenes	53±6.3 (3)	64±7.7 (4)	57±13.7 (3)

[a]Values indicate means±standard error (number of replicates).

hatched was independent of bacterial food source and post-feeding incubation time. We conclude that *C. elegans* continues to produce viable eggs over extended periods of feeding on pathogens known to cause foodborne diseases. Although this was suggested by observations on persistence of *C. elegans* in studies reported by others (Caldwell et al., 2003b; Kenney et al., 2004a), our study is the first to measure egg viability as influenced by feeding on enteric pathogens. In addition, results suggest that *C. elegans* may reproduce and persist on shed bacteria under conditions which support bacterial growth. Foodborne pathogens found in soils include both indigenous micro-organisms such as *L. monocytogenes* and other enteric pathogens known to survive outside their host (Cooley et al., 2003). They and others (IFT, 2001; Nicholson et al., 2000) showed that foodborne pathogens persist for extended periods in soil and on plant surfaces. By shedding viable cells of ingested *E. coli* O157:H7, free-living nematodes could contaminate soil and contribute to persistence of pathogens in agricultural environments.

Experiments were done to determine if *C. elegans* strain CB5584 that had fed on *E. coli* O157:H7 would disperse the pathogen in soil or soil amended with turkey manure compost. Tifton soil, a sandy loam soil collected from the lower Piedmont region in Georgia, was used. Moistened soil with or without added compost or supplemental exogenous food, i.e. *E. coli* OP50, was tested. Immediately after feeding on *E. coli* O157:H7, seven adult worms were transferred to soil and soil/compost. Nematode and bacterial populations were monitored for up to 7 days. Worms in various life stages were present 3 days after inoculation of unamended soil (12 worms/2.3 g, based on dry soil weight), soil amended with compost (16 worms/2.3 g), as well as soil to which *E. coli* OP50 had been added (81 worms/2.3 g) (Fig. 3). At 3 days, worm populations were significantly higher ($\alpha = 0.05$) in soils inoculated with *E. coli* OP50 than in soil with or without compost that was not inoculated with *E. coli* OP50. *C. elegans* persisted in soil containing *E. coli* OP50 throughout the 7-day test period; populations were 119 and 102 nematodes/sample at 5 and 7 days, respectively. Populations of *C. elegans* were not sustained unless *E. coli* OP50 was added as a supplemental food source. Thus, the addition of turkey manure compost to soil at a level of 0.66% did not provide sufficient nutrients to support growth of *C. elegans*. There is evidence that compost can increase nematode populations in some conditions (Bulluck et al., 2002), but this was not apparent in our study.

It was hypothesized that some of the *E. coli* O157:H7 ingested by *C. elegans* would be shed in soil. Experiments revealed that the number of *E. coli* O157:H7 shed by worms is dependent on the presence of *E. coli* OP50 in soil as well as the presence of nutrients in turkey

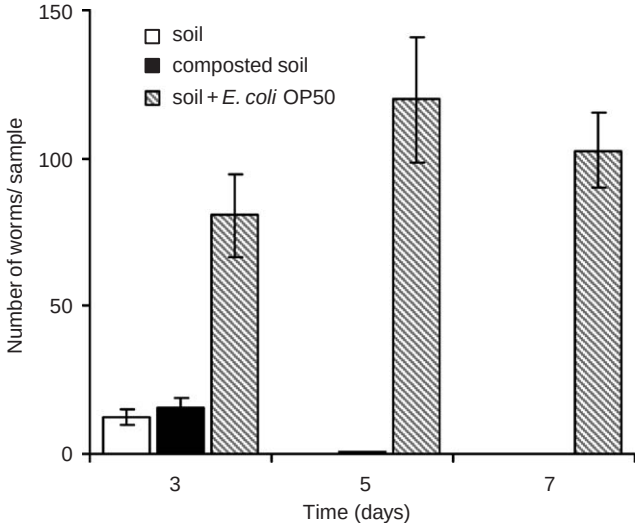

Fig. 3. Persistence of *C. elegans* in soil, soil amended with turkey compost, and soil inoculated with *E. coli* OP50.

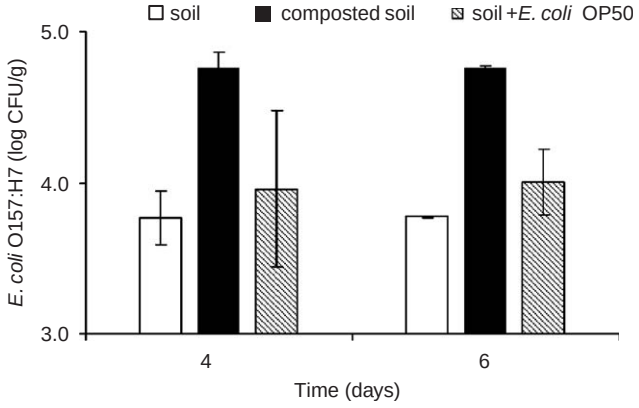

Fig. 4. Populations of *E. coli* O157:H7 detected in soil, soil amended with turkey compost, and soil inoculated with *E. coli* OP50 after inoculation with worms that had fed on *E. coli* O157:H7.

manure compost (Fig. 4). At 4 and at 6 days post inoculation, populations of *E. coli* O157:H7 were 4.79 and 4.71 $\log_{10}$ cfu g^{-1} of moist soil in compost-amended soil, which were significantly greater ($\alpha = 0.05$) than populations in unamended soil or soil inoculated with *E. coli* OP50. Thus, it appears that *C. elegans* shed *E. coli* O157:57 in all soil systems tested but the extent of contamination is greatest in soils amended with turkey manure compost. Populations of *E. coli* O157:H7 were not significantly different ($\alpha = 0.05$) at 4 and 6 days for soil, soil amended with compost, or soil inoculated with *E. coli* OP50. These observations suggest that nematode-mediated dispersal of *E. coli* O157:H7 in agricultural soils could occur.

Application of raw or improperly composted manure to soil in which fruits and vegetables are grown may introduce foodborne pathogens that persist for several months. Jiang et al. (2002) reported that *E. coli* O157:H7

survived for up to 231 days in manure-amended autoclaved soil held at 21 °C. *E. coli* O157:H7 has been reported to persist for 25–41 days in microcosms of fallow soils and for 92 days on alfalfa roots in silty loan soil (Gagliardi and Karns, 2002). Our study focused on determining the potential role free-living nematodes may play in enhancing persistence and distributing enteric pathogens in soil. The observation that *C. elegans* sheds *E. coli* O157, *S.* Poona, and *L. monocytogenes* for hours or even days following ingestion has important implications for the spatial and temporal distribution of pathogenic bacteria in soil. Measurements of nematode movements in soil are difficult and relatively little information is currently available. Dusenbery (1987) reported that free-living nematodes generally move about ten times faster than plant parasitic forms. The reported rate of movement for the latter is ca. $10\,\mu m\,s^{-1}$. Assuming that $100\,\mu m\,s^{-1}$ is an approximate rate for movement of *C. elegans*, worms traveling along a linear path could move 0.3–0.4 m during the 5 h over which they shed foodborne pathogens. If viable cells are shed for 24 h or more after ingestion, as is suggested in some studies (Caldwell et al., 2003b; Kenney et al., 2004), pathogens could be dispersed in soil over distances of several meters. The patterns of movement of foraging nematodes are complex; however, and the distances between ingestion and dispersal may be shorter than those predicted by worms following a linear path. Young et al. (1998) demonstrated that *C. elegans* readily moves a distance of 4 cm in moistened columns of sand over a period of 18 h. Boyd et al. (2001) reported a movement rate of $2.9–4.1\,\mu m\,s^{-1}$ (ca. $1.26\,cm\,h^{-1}$) at 20 °C for *C. elegans* on an agar surface. Although this rate of movement is less than that predicted by Dusenbery (1987), it would still allow for dispersal of worms at a distance over 6 cm during a 5-h period. Crowder et al. (2001) reported that *C. elegans* can move at a rate of $0.14\,mm\,s^{-1}$ (ca. $50\,cm\,h^{-1}$) on agar, without specifying the temperature. Assuming locomotion was determined at 20 °C, the incubation temperature used in our study, worms could disperse over a distance greater than 1 m within 5 h. Regardless of the rate of movement in soil, we conclude that *C. elegans*, and perhaps other free-living nematodes, may play a significant role in dispersal of bacteria, including foodborne pathogens, within the soil rhizosphere.

The observation that a free-living nematode, i.e. *C. elegans,* can disperse *E. coli* O157 in the soil has important implications for the persistence and distribution of foodborne pathogens. Bacteria naturally occurring in soil, as well as those that enter soil as a result of application of manure, compost, irrigation water, or runoff water, may exist in isolated pockets. Nematodes could contribute to the extinction of bacteria in one micro-environment or to their persistence in the general environment by vectoring cells to another favorable location. Increased persistence and movement of pathogens by nematodes could facilitate the subsequent transfer of these bacteria from the soil to the surface of preharvest fruits and vegetables. The work on internalization of plant tissues by foodborne pathogens (Cooley et al., 2003; Gandhi et al., 2001; Solomon et al., 2002; Wachtel et al., 2002) indicates that enteric pathogens in the root zone of plants may behave as passive endophytes. Studies should be done to determine if *C. elegans* and other free-living nematodes ingest, colonize, and disperse foodborne pathogens under field conditions used to grow fruits and vegetables.

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